ABSTRACT

Thermophilic cellulolytic enzymes can efficiently hydrolyze cellulose and covert glucose into bioethanol efficiently as compared to the mesophilic enzymes. Therefore, it is important to identified and isolated thermostable novel cellulolytic enzymes. A thermophilic bacterium *Caldicellulosiruptor sp.* ATCC BAA-1888/DSM 6725 has good potential to produce cellulases of industrial importance. Genomic DNA obtained from a thermophilic strain *Caldicellulosiruptor sp.* and examined for the presence of sequences encoding exo-cellobiohydrolase (Cbh). A pair of primers is used to amplify Cbh gene from genomic DNA of *Caldicellulosiruptor sp.*, Cbh gene consists of 1,644 bp, which encodes 547 amino acid residues with theoretical molecular weight of 61.44 kDa. The amplified Cbh gene is clone initially in pTZ57R/T cloning vector and then sub-cloned into an expression vector of pET-21(a)+, using ligated product to transformed competent cells of *Escherichia coli* BL21 CodonPlus, as a mesophilic expression host. Heterologous Cbh protein expression is analyzed by using SDS-PAGE, and a prominent band of 61.44 kDa was observed. Optimum recombinant expression of Cbh protein is observed using 0.5 mM IPTG concentration after 6 hour incubation at 37°C with a shaking speed of 200 rpm. Cbh enzyme has exhibited great affinity with various substrates like CMC, Avicel and pNPC. The maximum activity is observed against pNPC substrate. Enzyme was optimally active at pH 6.0 and at temperature 70°C. The results from this study indicate that of *Caldicellulosiruptor sp.* is the promising candidate for thermostable novel cellulases. The recombinant Cbh enzyme could be used efficiently in saccharification process and has several industrial applications.